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THE ANIMAL PROTEIN FACTOR

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THE ANIMAL PROTEIN FACTOR

By RUTH WOODS

Poultry science is one of the most important fields of applied nutrition. For that reason, possibly more than any other, it is also one of the most prolific sources of new findings in nutrition research. Low fertility, poor hatchability of eggs and high mortality of baby chicks are all serious economic problems. Their solution, in large part, is often dependent upon fundamental nutrition information. The practical investigation of these and other related problems in poultry science has contributed, more than once, to the discovery of new vitamins or related nutritional factors. Vitamin K, biotin, pyridoxine and pantothenic acid are some of the examples illustrating the indebtedness of nutrition research to poultry science.

During the past few years, investigations in poultry science have culminated in the discovery of a new nutritional factor (or complex of closely related factors). The new factor, as yet unidentified, is found in close association with animal proteins, but is not itself a protein. Referred to in poultry science literature as the "animal protein factor" and the "chick growth factor," it has also been named "zoopherin" (a factor needed to carry on animal species) by research investigators who have found it to be of equal importance in rat nutrition. The indispensability of the new factor in both poultry and rat nutrition may be considered a good indication that it will eventually be found to have a role in human nutrition as well.

Indeed, as this issue goes to press strong evidence in favor of this possibility is presented: The announcement has been made that the newly isolated crystalline human anti-pernicious anemia factor, vitamin B_{12}, may be identical with or closely related to the animal protein or chick growth factor.

Early Studies with Vegetable Proteins:

One of the most widely used protein supplements in poultry feeds, especially in recent years, is soybean meal. Soybean meal, particularly when heat-treated, is known to be a superior protein and a good source of essential amino acids.* Nevertheless, a number of conflicting reports have appeared during the past fifteen years concerning the adequacy of poultry diets containing soybean meal as the only source of protein. One of the earliest observed effects of such diets was a marked decrease, especially

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**cf. Borden's Review of Nutrition Research,* June, 1944, "The Nutritive Value of Soybeans."

in the winter months, in the hatchability of eggs produced by hens fed these diets (1, 2, 3, 4). That this decrease in hatchability was not due to the absence of such known hatchability factors as riboflavin or manganese was demonstrated by several workers (4, 5). The defect was found to be remedied by exposure of the hens to sunlight (6), or by complete or partial replacement of the soybean meal with meat scraps (4, 6).

Because these observations on decreased hatchability on soybean diets were not universally confirmed by investigators in poultry nutrition, the problem was investigated anew by Whitson, Titus and Bird (7) at the U. S. Agricultural Research Center in Beltsville, Md. In a preliminary study with a diet containing 33% soybean meal, 47% corn and adequate vitamin and mineral supplements, they had found a 61% hatch of the fertile eggs during November to the end of February and an 84% hatch during the period April 19 to August 9.

In a follow-up study some years later, these workers reported results of a forty-week experiment in which laying hens were fed diets consisting of corn, heated soybean oil meal and alfalfa leaf meal supplemented with vitamin and mineral concentrates. As the level of soybean oil meal was increased from 0 to 40 per cent (in increments of 10 per cent), the hatchability of the eggs produced decreased significantly in spite of the presence of adequate amounts of the dietary essentials known to affect hatchability (Table 1). These high levels of soybean oil meal, however, did not have an adverse effect on egg production, body weight or egg size.

Table 1—Egg Production and Hatchability

Diet			Egg production, %			Hatchability				
	Description					By Pens				
No.	Grain, %	Soybean meal %	By Pens		By Diets	No. fertile eggs	% Hatch	No. fertile eggs	% Hatch	By Diets % Hatch
	corn									
296	87	0	30.4	30.4	30.4	677	89.7	631	83.2	86.4
297	77	10	46.8	46.9	46.8	1176	82.9	1124	82.8	82.8
298	67	20	53.1	47.1	50.1	1472	77.5	1121	79.8	78.6
299	57	30	43.9	53.2	48.5	1024	68.1	1197	70.8	69.4
300	47	40	50.6	51.2	50.9	1181	65.3	1279	64.7	65.0
	1943 wheat									
301	67	20	52.0	51.6	51.8	1178	84.0	1177	83.2	83.6
	1937 wheat									
302	67	20	51.5	51.1	51.3	1073	85.6	1130	86.5	86.0
303	67*	20	57.1	54.3	55.7	1044	90.4	1055	87.2	88.8
Least significant difference (19:1 odds)					5.0					5.0

* Plus 10 mg. alpha tocopherol per lb. of feed.

In another experiment by these workers it was found that wheat supplementation of soybean meal (20%) was more effective in increasing hatchability than was supplementation with corn. The seasonal variation in hatchability was found to occur regardless of the type of diet fed.

In view of their findings Whitson *et al* concluded that there is at least one other aspect of the problem of low hatchability aside from the known effects of riboflavin or manganese deficiency. They postulated that either soybean meal has a direct depressing effect on hatchability or that wheat or high levels of corn supply an unknown dietary essential.

Coincident with these studies, Whitson *et al* (7a, 8) conducted some experiments designed to test the effect of dried cow manure supplements on both egg production and hatchability. These experiments were based on earlier reports by Hammond (9, 10) that the feeding of dried cow manure increased the rate of growth of chicks and stimulated comb development. The latter effect was attributed by Riley and Hammond (10a) to the presence of an androgenic (male) hormone present in cow manure. On the basis of these studies it was thought possible that the growth stimulating factor in cow manure might also enhance egg production and hatchability, while the androgenic factor would obviously inhibit these functions. Accordingly, Whitson *et al* (8) fed supplements of dried cow manure to hens on an adequate diet and found a marked depression of egg production as anticipated. When the cow manure supplement was treated to destroy its androgenic potency, no decrease in egg production was found.

The basic all-mash diet (containing meat protein and alfalfa meal) supported good hatchability with or without the androgen-free cow manure supplement. When, however, the treated cow manure supplement (8%) was added to a diet high in soybean meal and devoid of both animal protein and alfalfa, hatchability was significantly improved and the usual seasonal variations were largely eliminated.* Growth stimulation was also noted. Whitson *et al* concluded, therefore, that cow manure contains an unidentified factor which permits good hatchability and growth on a vegetable protein diet. Whether or not this hatchability factor was identical with the chick growth factor of Hammond remained to be seen. From their observations, Whitson *et al* (7a) concluded further that the unidentified factor was neither a protein nor any of the chemically characterized vitamins.

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*In a recent paper published in May of this year, Groschke *et al* (8a) confirmed these earlier studies in an experiment of unusually long duration. They found that addition of the growth factor in cow manure increased hatchability and completely eliminated seasonal variations regardless of exposure to sunlight.

The Search for the Hatchability Factor:

The demonstration of a new hatchability and growth factor in dried cow manure stimulated an investigation as to whether this factor might be present in other, more widely used feedstuffs. At the same time an attempt was made to obtain some knowledge of the distribution of this factor as a clue to determining its properties and identity. Studies along these lines were reported by Bird *et al* (11) two months after the work of Whitson's group. They used a high soybean (30%) basal diet supplemented with each of the following: Cow manure, sardine meal, corn gluten, dried skim milk, wheat (replacing corn in the high soybean basal diet), butyl fermentable solubles, manganese sulfate, soybean oil meal prepared by hydraulic instead of expeller process, soybean oil meal prepared by extraction instead of expeller process, and the basal diet minus alfalfa meal. The non-supplemented 30% soybean basal diet served as a negative control; a basal diet containing 10% sardine meal as the only source of protein served as the positive control. Composition of control diets is given in Table 2. Effect of these and of the supplemented diets is summarized in Table 3.

As had been previously reported, the 30% soybean meal diet resulted in low hatchability. During a period of 43 weeks only 66% of the fertile eggs hatched as compared with an 84% hatch of fertile eggs of hens on the positive control diet containing sardine meal as the only protein supplement.

The deficiency of the basal soybean diet was corrected by the addition of 5% cow or steer manure, 10% sardine meal, or 10% dried skim milk. Response to the other supplements was either negative or doubtful (Table 3).

Other good sources of the hatchability factor recently reported include rendering plant meal, packing plant meal, herring fish meal (11a).

An unexpected finding during the course of these studies was the fact that the high soybean meal diet impaired not only hatchability but had a marked effect on the viability of the chicks hatched. During the first week

Table 2—Composition of Diets

Ingredient	Diet 311	Diet 312
Ground yellow corn	57.0	78.3
Alfalfa meal	5.0	5.0
Sardine meal	—	10.0
Soybean oil meal	30.0	—
Steamed bonemeal	4.2	3.2
Limestone flour	2.3	2.0
Butyl fermentation solubles (250 micrograms riboflavin per gm.)	0.5	0.5
Manganized salt (containing 6% $MnSO_4$)	0.5	0.5
Iodized salt*	0.2	0.2
Vitamin A and D oil**	0.3	0.3

*100 parts NaCl, 0.3 KI, 0.4 $Na_2S_2O_3 \cdot 5H_2O$, 3.3 $CaCO_3$, 6 cornstarch.
**Contained 2000 U.S.P. units of vitamin A and 400 A.O.A.C. units of vitamin D per gram.

of life, from 16 to 38% of the newly hatched chicks died as compared with 1 to 11% mortality for the offspring of hens fed the positive control (sardine meal protein) diet. Those supplements to the hens' diets which improved hatchability (sardine meal, cow manure, skim milk) also improved the viability of the new chicks. Adding the supplements to the chicks' diet, however, had no effect in improving viability.

The fact that a cereal-soybean meal-alfalfa ration for chicks is deficient in an essential unidentified factor (or factors) present in meat, fish meal and dried skim milk was confirmed by a number of investigators (1, 2, 4, 5, 12, 13, 14, 14a, 14b). A similar fat soluble factor in meat scraps and liver paste was reported by McGinnis and coworkers (15). This latter factor was, like the others, not present in yeast and was reported to be different from choline. Bethke *et al* (16) confirmed the fact that neither choline nor methionine supplements (two factors sometimes deficient in soybean meal) (17) improved hatchability on the deficient diet.

In contradiction to these findings, Mishler and coworkers (18) at the Purdue University Agricultural Experiment Station reported that the deficiencies of a corn-soybean meal diet with respect to chick growth could be overcome by the addition of riboflavin, pantothenic acid, nicotinic acid and choline supplements. They concluded tentatively that animal protein supplements are not essential in the chick ration for rapid growth.

In a subsequent study, Mishler *et al* (19) found that the addition of condensed fish solubles to their basal corn-soybean meal ration containing

Table 3—Effect of Diet on Hatchability

No.	Diet	Hatchability, % of fertile eggs					
	Modification of diet 311	1–23 weeks			1–43 weeks		
		Series 1	Series 2	Mean	Series 1	Series 2	Mean
311	None	65	67	66	70	63	66
314	+5% cow (or steer) manure	80	80	80	82	83	82
315	+10% sardine meal	79	72	75	81	74	78
316	+15% corn gluten feed	61	64	62	—	—	—
317	+10% dried skimmilk	78	79	79	79	78	79
318	Wheat replacing corn	73	70	71	—	—	—
319	+0.5% B.F.S.* (1% total)	71	74	72	72	74	73
320	+0.025% $MnSO_4$ (0.05% total)	67	68	67	64	69	67
321	Hydraulic replacing expeller soybean oil meal	72	82	77	—	—	—
322	Extracted replacing expeller soybean oil meal	74	78	76	—	—	—
323	Minus alfalfa meal	54	70	62	59	77	68
	Modification of diet 312						
312	None	84	86	85	84	84	84
313	+5% steer manure	77	84	81	79	85	82

* Butyl fermentation solubles, 250 micrograms riboflavin per gm.

the four vitamin supplements, produced a marked supplementary growth effect. On the basis of these observations, they concluded that fish solubles appear to supply an essential unidentified factor or factors not present in the vitamin-supplemented vegetable protein ration.

The Nature of the Chick Growth and Hatchability Factor:

By 1946 the combined researches on the various investigations into the nature of the chick growth factor revealed that it was a vitamin-like material found in close association with animal proteins. The various experimental diets were completely adequate with respect to the essential amino acids for chicks (20), all of the chemically indentified vitamins (7a, 21) and all of the non-chemically identified known chick growth factors (22) (L. casei factor, factor U, factors R and S, Vitamins B_{10} and B_{11} and folic acid*). These facts established the chick growth factor as a new, unidentified factor in poultry nutrition.

In an attempt to identify the new factor, Rubin and Bird (23) succeeded in extracting and concentrating the factor from cow manure. Potent concentrates supported growth when fed at levels of 3.75 to 7.5 milligrams per 100 grams of diet (as compared with 8,000 mg. per 100 g. of cow manure). Examination of its chemical properties showed it to be heat stable, moderately water soluble, fat insoluble and non-dialyzable through cellophane, thereby indicating it to be a large molecule. Rubin and Bird made the further observation that the growth factor can be transmitted from the hen through the egg to the chick. This was evidenced by the fact that chicks from hens fed the cow manure or fish meal concentrates were able to grow well on the basal diet devoid of the chick growth factor.

In a subsequent report, Rubin, Bird and Rothchild (24) demonstrated the presence of the chick growth factor in hen feces as well. The factor was found in the feces of hens on diets free of this substance, thereby indicating some degree of intestinal synthesis by the hen. The feces of growing chicks did not contain measurable amounts of the growth factor.

Transmissability of the Chick Growth Factor: The importance of the breeder diet of hens with respect not only to hatchability, but viability and growth of chicks is emphasized by the findings of independent investigators (23, 25, 26, 27) that the growth factor is transmitted to the egg, stored and eventually supplied to the tissues of the chick.

The actual distribution of the growth factor in eggs was determined by Rubin and Bird (26). The factor appeared in eggs, chiefly in the yolk, of hens receiving it in their diet. Eggs laid by hens fed a diet deficient in

*Subsequently synthesized and found to be not identical with the chick growth factor.

the growth factor were, in turn, relatively deficient in the factor. A sufficient amount of the growth factor found in eggs from hens on an adequate diet was found to be provided by supplying chicks with two yolks per kilogram of feed.

A New Dietary Essential for the Rat:

Coincident with the extensive studies on the new factor(s) necessary for reproduction (hatchability), growth and viability in chicks, other investigators were reporting, independently, similar findings with rats. In 1942, Ross and coworkers (28) demonstrated that a basal ration of corn, soybean oil meal, minerals and alfalfa meal was inadequate for normal growth, reproduction and lactation in the rat. These observations were confirmed by Cunha (29). Shortly thereafter Spitzer and Phillips (30) reported the existence in liver powder, acid-treated casein plus choline or fish meal of an unidentified factor necessary for reproduction and lactation in rats maintained on such a diet. This coincided with evidence presented by Cary and Hartman (31, 32) for the existence of an unidentified nutrient (X) required by the rat for growth, reproduction and lactation on diets containing a highly purified casein as the source of protein.

Zoopherin: In January of this year a very thorough report on one of the new rat factors was presented by Zucker and Zucker (33). These investigators fed rats a purified diet well fortified with protein and all known essential vitamins; supplements of yeast were also provided as a source of unidentified B complex vitamins. Female rats kept on this diet exhibited normal reproductive abilities and successful lactation. The young born of such females, however, exhibited a high incidence of mortality soon after weaning if kept on the basal diet deficient in the postulated rat factor. In a typical experiment 30 out of 66 weanling rats died; those which survived grew slowly and at 20 weeks were 40% below normal weight. There was a tendency for individual litters either to survive entirely or to all succumb, indicating possible genetic differences in requirement, storage or synthesis of the essential factor. Similar genetic differences were observed in chicks by Bird *et al* (34). In addition to severe growth failure, other symptoms included abnormally high blood urea and a marked drop in white cell count (leukopenia). The immediate cause of death appeared to be hemorrhage in the upper part of the stomach. Postmortem findings included shrunken spleen and slight enlargement of the liver and kidneys of a type unrelated to choline deficiency.

The deficiency syndrome was prevented or cured by supplements of crude casein, fish solubles or a liver extract. Plant proteins, including yeast, were ineffective.

The new factor, tentatively named zoopherin, was found to be soluble in water, dilute acid, alkali and alcohol and fat insoluble. Unless it can occur as a lipid-soluble compound or conjugate, the authors point out, it is not the same as the fat soluble "animal protein factor" of Heuser (15, 35) and Johnson (36) and associates. It also has been differentiated from such related factors as strepogenin, factors R and S, vitamins B_{10} and B_{11}, factor U and the so-called monkey factor, as well as similar factors postulated by Sporn *et al* (37), Bowland *et al* (38) and others.

Mechanism of Behavior of Chick Growth Factor:

Because of the deficiency symptoms induced in chicks on high soybean meal diets the possibility existed that soybean meal might contain an inhibiting substance which interfered with embryonic development (hatchability) and growth. Raw soybean oil meal is known to contain at least one such growth inhibitor, a substance which interferes with the action of the digestive enzyme, trypsin (39, 40). The trypsin inhibitor was ruled out, however, because it is heat labile and would, therefore, be destroyed in heat-treated soybean meal. Furthermore, the trypsin inhibitor is a protein and therefore would not be likely to be absorbed through the intestine.

Possible Role of Methionine: Methionine has been reported by many investigators to be effective in improving the nutritional value of both raw and heated soybean meal.* Bird *et al* (34) showed that the addition of methionine to a high soybean diet produced as good growth in chicks as did the cow manure factor *provided this factor had been present in the diet of the hens.* Methionine was less effective when fed to chicks from hens whose diet was deficient in the cow manure factor. Inasmuch as the basal diet apparently provided adequate quantities of the essential amino acids, the reason for the activity of methionine remains obscure. Some interrelation of the growth factor and methionine is indicated. As suggested by Rubin and Bird (41) the growth factor might conceivably facilitate the liberation of methionine from soybean protein in the digestive tract; it might function in the metabolism of methionine after absorption; or it might have some entirely different metabolic function which could be partially performed by methionine.

**cf. Borden's Review of Nutrition Research,* June, 1944, "The Nutritive Value of Soybeans."

In a series of experiments designed to study the role of methionine, Rubin and Bird (41) found that methionine supplements had no effect in counteracting the detrimental effects of an excessively high (70%) soybean meal diet. The chick growth factor, however, was successful in overcoming these effects. This would seem to indicate that the effects of soybean meal are not limited to interference with the liberation of methionine in the digestive tract.

Rubin and Bird have postulated that soybean meal (and possibly other plant proteins) contains an inhibitor of growth which is stable to heat and which produces an effect not confined to the digestive tract. The growth factor of cow manure apparently overcomes the effects of this heat-stable inhibitor. (It has no effect on the heat-labile trypsin inhibitor of soybean.)

On the basis of the work by Cary and Hartman, however, another explanation might be simply that the need for the animal protein factor is intensified as the quantity of protein (as, for example, soybean) is increased in the diet. These workers have demonstrated that certain animal proteins, such as egg white, do not contain the essential factor, and that other animal proteins rendered devoid of the factor by chemical treatment produce inferior growth and even toxicity when fed at sufficiently high levels.

Trypsin-Methionine-Growth Factor Interrelationships: Although the possible interrelationships among these three factors are still somewhat obscure, it may be of interest to point out some of the existing possibilities. According to Melnick *et al* (42) the poor utilization of raw soybean protein is due to inhibition of trypsin which results in the retarded liberation of methionine. Heated soybean provides more available methionine which is utilized both for structural purposes and, to some extent, as partial substitute for the growth factor. Even heated soybean protein, however, contains barely adequate, or slightly inadequate, quantities and thus responds to further methionine supplementation. More effective supplementation is obtained with the growth factor.

Identity of the New Chick Growth Factor:

Early this year, more than 20 years of intensive research by numerous investigators in another field of investigation, culminated in the dramatic announcement that the long-sought, mysterious anti-pernicious anemia factor* of liver has finally been isolated in pure, crystalline form (43, 44). Shortly after this material became available, Ott, Rickes and Wood (45)

**cf. Borden's Review of Nutrition Research,* March, 1946, "Nutritional Milestones in the Anemias."

announced that it possesses "animal protein factor" activity for the chick. They found that crystalline vitamin B_{12}, when added to purified basal diets with 40-70% soybean meal as the only source of protein, exerted animal protein factor activity in chicks from hens on all-plant ration such as described by Rubin and Bird (23). Chicks responded to various supplements known to be sources of the animal protein factor and also to vitamin B_{12} in doses as small as 6 micrograms per kilo of diet. The authors concluded that since the crystalline substance elicited growth responses comparable to crude sources of the animal protein factor, it is possible that vitamin B_{12} is identical with or closely related to this factor.

The chemical nature and structure of the new vitamin B_{12} has not yet been completely determined. It is known to contain three molecules of phosphorus and some nitrogen, but no sulfur. Its probable molecular weight is between 1550-1750. Most significant finding to date regarding its composition is the announcement, made this summer, that vitamin B_{12} is a cobalt complex (46, 47). This emphasizes anew the role of cobalt in animal and human nutrition, especially with reference to its anti-anemia properties.

Vitamin B_{12} has been used clinically with marked success in the treatment of human pernicious anemia and other anemias. If this material should indeed be identical with one or more of the various chick growth and hatchability factors or the rat factors, it will demonstrate again the close relationship between the nutritional requirements of animals and man.

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